Title Page

Rituximab is Ineffective for Treatment of Fatigue in Primary Biliary Cholangitis: A phase-2 Randomised Controlled Trial

Author Names:

*Amardeep Khanna[1,2,4], *Laura Jopson[1,2], Denise Howel[3], Andrew Bryant[3], Andrew Blamire[1,2], Julia L Newton[1,2], David E Jones[1,2,4]

* Denotes equal contribution

Affiliations:

1. Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, UK.

2. NIHR Biomedical Research Centre, Newcastle University, Newcastle upon Tyne, UK.

3. Institute of Health & Society, Newcastle University, Newcastle upon Tyne, UK.

4. Freeman Hospital, Newcastle upon Tyne, UK.

Authors' email addresses:

Laura.jopson@newcastle.ac.uk; amardeep.khanna@newcastle.ac.uk;

denise.howel@newcastle.ac.uk; andy.bryant@newcastle.ac.uk;

Andrew.blamire@newcastle.ac.uk.; julia.newton@newcastle.ac.uk.;

david.Jones@newcastle.ac.uk

Corresponding Author:

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/hep.30099

Professor David Jones FRCP, PhD. Institute of Cellular Medicine, 4th Floor William Leech Building, Medical School, Framlington Place, Newcastle upon Tyne, NE2 4HH. Tel: 0044-191 2088782, Email: david.jones@newcastle.ac.uk

Conflict of interest statement: Prof David E Jones report grants and personal fees from Intercept, grants from Pfizer and others from GSK, Novartis, Cymabay, FFPharma and Shire during the conduct of the study. All other authors declare no conflict of interest.

Author's contribution: All Authors contributed equally to drafting this manuscript.

Clinical Trial Number: NCT02376335

Keywords *(5 keywords, not in title):* Anti-mitochondrial antibodies, Anaerobic threshold, Anti-PDC antibodies, PBC-40, Euclidian Norm minus One.

Footnotes Page

Contact Information: Professor David Jones FRCP, PhD. Institute of Cellular Medicine, 4th Floor William Leech Building, Medical School, Framlington Place, Newcastle upon Tyne, NE2 4HH.

List of Abbreviations:

AMA	Anti-mitochondrial antibody
ENMO	Euclidian Norm minus One
Ig	Immunoglobulin
PBC	Primary Biliary Cholangitis
PDC	Pyruvate Dehydrogenase Complex
SD	Standard deviation 5

Funding: This trial was funded by the Medical Research Council and NIHR Efficacy and Evaluation program (EME 10/90/03), Newcastle NIHR Biomedical Research Centre in Ageing and Long-term Conditions and a DoH subvention grant.

Abstract

Primary Biliary Cholangitis (PBC) is a chronic cholestatic liver disease. Half of patients experience debilitating fatigue which is currently untreatable. Previous studies have shown muscle bioenergetic abnormalities in PBC, including increased muscle acidosis with exercise linked to the anti-mitochondrial antibody (AMA) diagnostic of the disease, and reduced anaerobic threshold. In this study we addressed the hypothesis that fatigue in PBC is driven by muscle bioenergetic abnormality related to AMA, and that AMA reduction with B-cell depletion therapy will improve fatigue. In our single-centred Phase II randomised controlled trial (RCT) 57 participants aged ≥18 years with PBC and moderate or severe fatigue were randomized to receive 2 doses of either rituximab (1000mg) or saline (placebo). The primary outcome measure was fatigue severity assessed using the PBC-40 fatigue domain at 3 months. Secondary outcomes measures included patient-reported outcomes, immunological and bioenergetics disease parameters. Experimental outcomes included biochemical markers of disease severity. Improvement in fatigue score at 3 months was seen in both arms, with no significant difference (adjusted mean difference -0.9 95%CI -4.6 to 3.1). Little difference was observed in other patient reported outcomes or physical activity. Significant anaerobic threshold improvement was seen in the Rituximab group only but this was not associated with fatigue improvement. No treatment-emergent SAEs were seen.

Conclusions*:* Rituximab was safe over the 12 month study period but showed no evidence of effectiveness for the treatment of fatigue in PBC. Anaerobic threshold improvement was seen; potentially linking AMA with muscle bioenergetics dysfunction, however, this was not

related to improvement in fatigue. Rituximab had some evidence of a beneficial effect on alkaline phosphatase levels in this largely UDCA-responding, early disease stage cohort.

Introduction

PBC is a chronic liver disease characterized by loss of intra-hepatic bile ducts accompanied by progressive cholestasis (1). 50% of patients experience moderate or severe fatigue; a debilitating symptom affecting quality of life and contributing to social isolation and significant quality of life impairment (2-4). Fatigue, the symptom most frequently reported by PBC patients [2-4], can occur at any point in the disease course, and its severity is unrelated to liver disease activity or degree of liver damage and is not improved by current first or second line therapy (5, 6),(7) . Given its impact, and lack of response to therapy, increasing understanding of, and treatment for, fatigue has been highlighted by patients as a priority for research. There have, however, been only 2 placebo controlled trials with fatigue as the primary endpoint. Neither of these trials, exploring the use of Fluoxetine and Modafanil, showed evidence of benefit (8, 9). At the time of the design of this trial evidence was emerging to suggest that the anti-CD20 biological agent Rituximab was effective for the reduction of fatigue in a number of immune conditions, including Primary Sjogrens Syndrome, a condition which shows an association with PBC (10-13). This raised the question as to the possible value of Rituximab as a fatigue modifying treatment in PBC. The potential value was supported by a possible direct biological mechanism. PBC is characterized immunologically by the presence of AMA, a population of high titer autoantibodies directed at Pyruvate Dehydrogenase Complex (PDC), an enzyme complex which play a critical role in cellular bioenergetic function linking glycolysis and the Krebs cycle (14). Anti-PDC antibodies from PBC patients are highly effective, in vitro at least, at

blocking PDC function (15). Clinically, PBC patients exhibit both central and peripheral elements to their fatigue. The peripheral component, likened by patients to feeling that their "batteries are running down", is associated with inability to sustain repeat muscle contraction. Investigation of this phenomenon, using novel magnetic resonance spectroscopy approaches, revealed marked muscle acidosis with exercise, related to mitochondrial dysfunction, and a prolongation in the time taken for recovery of muscle acidosis following discontinuation of exercise which was related to fatigue severity (16). The degree of mitochondrial dysfunction was related to serum anti-PDC level (17). In separate approaches PBC patients have been shown to have lower anaerobic threshold levels than matched normal and cholestatic controls (18). Taken together, these observations point to the possibility of dysregulation of aerobic metabolism in muscle in PBC, with excessive or inappropriate utilization of the anaerobic lactate dehydrogenase pathway. The link between anti-PDC levels and mitochondrial dysfunction, and the capacity of anti-PDC to block PDC function led us to postulate that anti-PDC may be a driver for metabolic insult in PBC that this insult may contribute to fatigue and this effect may be reduced by Rituximab as a B-cell targeting drug. A pilot study completed in Canada had provided some evidence to support this contention (19).

The objectives of the current study were to assess whether Rituximab improved moderate or severe fatigue in patients with PBC, to assess the safety and tolerability of Rituximab in PBC and to assess the effect of Rituximab bioenergetics abnormality potentially linked to fatigue.

Methods

The protocol for this study has been published and the study design, criteria and procedures outlined in detail (20).

Subjects

Seventy-one participants aged ≥18 years with PBC and clinically significant fatigue (as defined by a PBC-40 fatigue domain score of >33 (mean + 2SD for a normal control population (4)) were screened at a single clinical center at Newcastle, led and managed by the Newcastle upon Tyne Hospitals NHS Foundation Trust. Fifty-seven participants were randomized in a 1:1 ratio to receive either Rituximab (1000mg) (n=28) or saline intravenous infusion on days 1 and 15 (placebo, n=29). The infusions were delivered in a double-blind manner using the same protocol. All participants had a clinical diagnosis of definite (meeting all 3 criteria) or probable (meeting 2 of 3 criteria) primary biliary cholangitis (cirrhosis) established using recognized epidemiological criteria:

a) Cholestatic liver biochemistry at disease outset (defined as elevation in the serum Alkaline phosphatase (ALP) level or gamma-glutamyl-transferase (GGT))
b) Associated autoantibody (anti-mitochondrial antibody or PBC-associated antinuclear antibody by immunofluorescence or anti-PDC, anti-Gp210 or anti-Sp100) at a titer of ≥1:40
c) Diagnostic or compatible liver biopsy

Patients in whom ALP and GGT return to normal with therapy retained their diagnostic status.

Inclusion & Exclusion Criteria

The study criteria are published in detail elsewhere. The key study characteristics were

Inclusion

- Clinically significant fatigue (PBC-40 fatigue domain score >33 (4))
- Presence of AMA at a titer of >1:40
- Hb >9g/L, absolute neutrophil count >1.5x10^9/L, platelet count > 50x10^9/L
- Bilirubin ≤ 50 µmol
- INR ≤ 1.5
- Child Pugh score < 7
- Adequate renal function; Cockcroft and Gault estimation > 40ml/min
- age ≥ 18 years
- Patient had capacity and provided written informed consent.

Exclusion

- Advanced or decompensated liver disease
- Aspartate transaminase (AST) / Alanine transaminase (ALT) 4 x upper limit of Normal (ULN)
- History or presence of other concomitant liver diseases
- Previous treatment with B-cell depleting therapy
- Previous history of aberrant response or intolerance to immunological agents or to other study agents
- Presence of clinically significant untreated intercurrent medical condition itself associated with fatigue including depressive illness
- Statin use (current or use within 3 months of enrolment)
- Ongoing participation in other clinical trials or exposure to any investigational agent 4

weeks prior to baseline or within < 5 half-lives of the investigational drug

- Intercurrent active or latent infection
- Intercurrent immuno-compromised state
- Malignancy (other than basal cell carcinoma) within the last 10 years

Study Design

This was a single centered phase II, double-blind, randomized controlled trial. Participants received two infusions on days 1 and 15 and were then followed-up at 3, 6, 9 and 12 months. The primary endpoint was improvement in fatigue severity at 3 months with extended follow-up (up to 12 months) to explore the stability over time of clinical or phenotypic change.

Randomization

Randomization was conducted by the Newcastle Clinical Trials Unit (NCTU) web-based system on a 1:1 ratio using random-permuted blocks with random block length. The randomization system generated a treatment number for each participant that linked to the corresponding allocated study drug. Patients could only be randomized into the study by an authorized member of research team, as detailed on the Study Delegation Log.

Experimental Intervention

The investigational medicinal product used in the clinical trial was Rituximab, 1000 mg IV (MabThera®). This product was approved by the European Agency for the Evaluation of Medicinal Products (MA number: EU/1/98/067/002) and license held by Roche Products

Ltd. The product was supplied as vials containing 500 mg of Rituximab at a concentration of 10mg/ml and stored between 2°C and 8 °C, in a secure location in their original packaging to avoid light damage. Supplies of Rituximab were labelled as investigational medicinal product (IMP). Patients randomized to receive Rituximab therapy were given treatment at the infusion rates recommended for rheumatoid arthritis (RA) patients (i.e. for the first infusion at an initial rate of 50 mg/hr.; after the first 30 minutes it could be escalated in 50 mg/hr. increments every 30 minutes, to a maximum of 400 mg/hr.). Second doses were infused at an initial rate of 100 mg/hr., and increased by 100 mg/hr. at 30 min intervals to a max of 400mg/hr (21). The control infusion was delivered in a double blind manner to participants under the supervision of a clinician (all clinical staff were blinded as to the study arm with the infusions prepared in centralized pharmacy facility and provided to the clinical research facility in the anonymized form. In line with recommendations for the administration of Rituximab in other conditions, patients received a conditioning regimen prior to the infusions of study medication or placebo. This conditioning regimen was administered 30 minutes prior to infusion of study medication and comprised: paracetamol 1g PO; chlorpheniramine 10mg IV; and methyl prednisolone 100mg IV. Conditioning was given to all participants to maintain the blind.

Outcomes

The **primary outcome** measure was the fatigue domain score from the PBC-40 questionnaire at 3 months. The PBC-40 is a fully validated patient-derived quality of life measure which includes a fatigue domain with a potential value range of 11 to 55 and higher values denoting worse fatigue)(22). The time course of the comparison between

intervention and control groups over the 12 month follow-up period was also assessed.

Secondary outcome measures included the following:

1) Other symptom severity assessed by the relevant domains of the PBC-40 (Itch, Cognition, Social, Emotional and Symptoms) (22). Anxiety and depression were assessed by Hospital Anxiety and Depression Scale (HADS) score (range 0-42) (23); daytime somnolence by Epworth Sleeping Scale (ESS) (range 0-24) (24); vasomotor autonomic symptoms using Orthostatic Grading Scale (OGS) (range 0-20) (25); functional status by Patient-Reported Outcomes Measurement Information System-Health Assessment Questionnaire (PROMIS HAQ) (range 0-100) (26), and cognitive functionality by Cognitive failure questionnaire (COGFAIL) (range 0-100) (27), where higher scores indicate worse outcome.
2) Fatigue diaries using both structured (quantitative) and unstructured (qualitative) methods of data collection undertaken 6 times during the study (for a week at baseline and during the first weeks of months 1, 3, 6, 9 and 12.
3) Physical activity monitoring using wrist worn tri-axial GENEActiv accelerometers (Activinsights Ltd., Cambridgeshire, UK). The accelerometer was worn continuously on the right wrist for a period of 7 days in free-living conditions. Patients were included in the analysis if they had worn the monitor for a minimum time period of 5 days (at least one days on the weekend). Only days with at least 22 hours of valid data were retained for analysis. Activity data are presented as Euclidian Norm minus One (ENMO) values for the per hour average of the whole assessment period, and for the highest activity 5 hours of the period. Higher values indicate greater activity levels.
4) Anaerobic threshold (AT) assessed using conventional Cardiopulmonary Exercising (CPX). Participants cycled on a stationary ergometer (Corival, Lode, and Nederland) at between

60-70rpm. Anaerobic threshold was determined using the computerized v-slope method at baseline and 12 week follow up.

5) Muscle bioenergetic function was assessed using Magnetic Resonance Spectroscopy at baseline and at 12 weeks using a 3T Intera Achieva scanner (Philips, Best, and NL). The protocol used for acquisition and analysis has been described in detail in published protocols(17).

6) Quantification and phenotyping of total B-cell populations and B-cell subsets was undertaken using a standard Fluorescence-activated cell sorting (FACS) based approach. Total B-cells levels in peripheral blood were evaluated using a direct immune-fluorescence reagent (Fast Immune CD19/CD69/CD45, BD Biosciences). Anti-PDC antibody total and individual isotype levels were studied on day 0 and at 12 weeks using a well-established Enzyme Linked Immunosorbent Assay (ELISA) developed within our research group.

Change in liver serum biochemistry was an **exploratory outcome measure**.

Statistical Analysis

The study was planned to detect a mean change in PBC-40 fatigue domain score of 5 units at 3 months follow-up (a difference demonstrated in our population-based studies to be associated with significantly higher levels of social function) (5). Previous studies had shown a standard deviation of 8 units and a correlation of 0.6 between baseline and follow-up, so using a power of 90% and a 5% significance level, this required outcome data from 35 participants per arm. A total of 78 participants (39 per arm) was planned to be recruited and randomized; assuming 10% attrition at 3-month follow-up. However, since recruitment was

slower than expected, trial recruitment was extended by 6-months and the power of the trial was reduced to 80% with a revised target sample size of 58 participants (29 per arm).

All analyses were performed on the intention-to-treat (ITT) population. If at least 50% of responses were present for all PBC-40 domains (and 80% for all other secondary questionnaires) then the median value for the completed items in the domain was ascribed to any missing items.

Baseline (BL) characteristics of the study population were summarized separately within each randomized group. The primary analysis was the PBC-40 fatigue domain: descriptive statistics were reported at each time point (baseline, 3, 6, 9 and 12 months) in both arms. PBC-40 fatigue domain scores were compared at 3 months between intervention and placebo group using multiple linear regression using a backward stepwise procedure (setting significance level at 10%) with adjustment forcing inclusion of baseline PBC-40 fatigue score, then selecting from age in years, UK PBC risk model score at 10 years prediction and patient location (managed by Newcastle center for at least one year or not) at baseline. The results were reported as an adjusted difference in means with a 95% confidence interval. The same approach was used when secondary outcomes were assessed. Bootstrap estimation was used throughout as it was not clear that the distributions were normal. The time course of the comparison between intervention and control groups over the 12 month follow-up period using time points above was assessed for PBC-40 fatigue domain using repeated measures Analysis of variance (ANOVA). The above analyses for PBC-40 fatigue score were repeated for secondary outcomes. Descriptive analyses were reported for biochemical and immunological measures. Additionally repeated measures ANOVA up to 12 months was carried out for bilirubin and serum alkaline phosphatase outcomes. The correlation between

degree of change in anaerobic threshold and change in fatigue over 3 months from baseline was also reported using Pearson's correlation coefficient. The statistical software package StataIC (version 14^{o}) was used for all analyses.

Results:

Subjects Disposition and Baseline Characteristics

Recruitment to the trial took place between October 2012 and October 2015. Seventy-one participants were recruited into the trial. 57 were randomized, 7 were screen failures, 6 patients withdrew and there was one death (unrelated to participation). Rates of attrition were low, with 50 participants staying in the trial for the full 12 months follow-up; 55 provided outcome data at 3 and 6 months and 54 at 9 months with two participants being lost to follow-up and one withdrawn. (**Figure 1**). Patients' baseline characteristics are presented in **Table 1.** Significant levels of both daytime somnolence (as assessed by the ESS) and vasomotor autonomic dysfunction (as assessed by the OGS) were seen at baseline in both the Rituximab and the placebo groups, with mean values exceeding the recognized values for clinical significance for both (10 for ESS and 4 for OGS; **Supplementary Table 1**). The mean age was 55 years old with the expected female predominance and all but one participant was white. In keeping with the study criteria, all participants exhibited significant fatigue.

Primary Outcome

Seventeen of the 27 (59%) Rituximab-treated patients and 10/28 (36%) of the placebo group achieved the pre-specific end-point of a drop of 5 or more units in the PBC-40. There

was no statistically significant difference in fatigue score at 3 months between Rituximab and placebo arms (adjusted mean difference -0.9 (95%CI - 4.6 to 3.1). However, improvement was observed in both arms (with mean score decreasing from 41.2 (Standard deviation SD=5.5) to 36.2 (SD=8.4) and 43.0 (SD=5.9) to 38.1 (SD=8.7) in the Rituximab and placebo arms, respectively (**Figure 2**, **Supplementary Table 1**). There was no significant difference between the two trial arms over the repeated assessments at 3, 6, 9 and 12 months (Trial arms F=1.81, P=0.18; Arms x Time points F=0.41 P=0.80) (**Supplementary Table 1)**. Fatigue diaries showed no statistically significant difference between the study groups (**Supplementary Table 1)**, including in the assessments at 1 and 2 months suggesting there was no fatigue signal which we were missing by using a 3 month end-point (data not shown).

Secondary Outcomes

Immunological analyses suggested that Rituximab was highly effective in mediating depletion of B-cells, its primary proposed mode of action (**Figure 3a**). No depletion was seen in the placebo arm. Complete depletion was maintained at 6 months with gradual repopulation to 50% of baseline by 12 months. Reduction in the levels of anti-PDC antibody was also seen in the Rituximab (but not placebo) group (**Figure 3b**) with peak reduction at 6 months and sustained at 9 months. No correlation was observed, however, between reduction in anti-PDC antibody level with Rituximab treatment and reduction in fatigue (data not shown). Reduction in total immunoglobulin (Ig) and, in particular the IgM fraction was observed. Again reduction was incomplete in the Rituximab treated group and absent from placebo (**Supplementary Table 2**).

For the additional patient reported outcome measures (PROMs) - ESS, OGS, COGFAIL, HADS, PROMIS-HAQ and 5 non-fatigue domains of the PBC-40, the unadjusted and adjusted differences in mean scores between trial arms showed little difference at 3 months, the 95% CIs were generally wide but there was no suggestion that the results were consistent with any clinically important differences (**Supplementary Table 1**). In contrast to fatigue, no notable improvement was seen in either group for the other measures. Any potential placebo effect was therefore restricted to fatigue. Physical activity levels (presented as Euclidian Norm minus One (ENMO) values) differed little between arms at 3 months: the adjusted mean ENMO levels were slightly lower in the Rituximab arm, but there was little indication of any meaningful difference (**Supplementary Table 2**).

We used a combination of muscle MR spectroscopy and anaerobic threshold CPX to explore muscle bioenergetic function. In keeping with previous reports, baseline anaerobic threshold was found to be low in the PBC patient group. Anaerobic threshold values at 3 months rose significantly from baseline with Rituximab but not with placebo (adjusted difference 1.41, 95% CI 0.03 to 2.80; **Figure 4a, Supplementary Table 3**). There was, however, no apparent correlation between change in anaerobic threshold and change in fatigue over 3 months from baseline (Pearson's correlation coefficient 0.12 (95% CI: -0.18 to 0.40)). The minimum pH following the specific exercise task was highly variable amongst the PBC patients, with substantial acidosis seen in some patients. There was no statistically significant difference in minimum muscle pH following exercise between arms (**Figure 4b, Supplementary Table 3**). No significant reduction in the time taken to recover to baseline pH after exercise, and no reduction in the "area under the curve" for pH (a factor combining the degree of acidosis and the length of time taken to recover to baseline and

an estimate therefore of degree of muscle intracellular acid exposure) was seen in either arm.

Exploratory Analysis

RITPBC though not designed or powered to explore the impact of the drug on liver injury; did provide some insight into the impact of Rituximab therapy in early disease which could inform future trials of disease modifying therapy. Baseline biochemical parameters were typical of early-stage PBC. Median alkaline phosphatase values were 136 and 131 u/L in the Rituximab and placebo groups, respectively. There were no significant differences between trial arms or in the interaction between arms over time in repeated measure ANOVA up to 12 months for bilirubin, but there were significant differences between trial arms (F=6.17, P=0.016) and in the interaction between arms over time (F=2.91, P=0.023) for ALP outcome (**Supp Table 4 and 5**). Median ALP fell from 136 to 106 u/L over 3 months in the Rituximab arm, whereas, the median ALP level rose from 131 to 137 u/L in the placebo arm. Ninety-three percent of patients in the Rituximab arm had a normal ALP at 3 months compared to 78% at baseline (65% from 61% in the placebo arm; **Supp Table 6**). All parameters progressively returned to baseline levels by 12 months of follow-up.

Safety

There were four serious adverse events (SAEs) during the course of the trial, none of which occurred in the active treatment group. One death from lobar pneumonia and Chronic Obstructive Pulmonary Disease (COPD) which occurred after consent, but prior to baseline

visit. One participant in the placebo arm was hospitalized for right optic neuritis and at a later date for left optic neuritis, but made a full recovery on both occasions. One participant in the placebo group was hospitalized for flare of chronic abdominal pain, but made a full recovery with opiate treatment.

Discussion

Fatigue is an important and complex clinical problem in PBC and is currently untreatable. Improvement in treatment is needed to enhance the life quality of patients. Improvement in fatigue with a mean reduction of over 5 units in the PBC-40 fatigue domain score (the predefined level for a clinically significant change) was observed in both the active drug and placebo groups. Gradual increase almost back to baseline levels was seen by 12 months. The lack of beneficial effect on physical activity level, an objective measure that has relevance to the subjective domain of fatigue would further support the view that there is no significant fatigue improvement with Rituximab in PBC. On the basis of our findings there is, therefore, no evidence to support the use of Rituximab as a treatment for fatigue in otherwise unselected PBC patients, and there is no evidence to suggest that B-cell related immune-phenomena play a significant role in fatigue pathogenesis in the disease. Levels of patient interest were, however, high amongst patients, as was retention within the study, both indicating a high degree of patient acceptability and supports the view that trials of therapy targeting symptoms in PBC are deliverable and highly acceptable to patients.

B-cell depletion was complete in all the Rituximab treated patients, excluding a technical drug failure, with progressive re-constitution over the year of follow-up. Over a period of 12 months, Rituximab was found to be safe. No SAEs were observed in the active drug group. In particular, no worsening of liver function was observed. This is in keeping with other reports of the agent in PBC and in contrast to the observation of worsening of liver function in murine models of PBC treated with anti-CD20 (19, 28, 29). This divergence of effects is likely to reflect the limitations of current murine models of PBC and challenges their relevance to therapeutics development. Rituximab had a benign safety profile in this trial. It is important to note, however, that follow-up was only for 12 months meaning that the possibility of late adverse sequelae cannot be excluded.

Potential explanations for the apparent reduction of fatigue severity in the placebo arm include the placebo effect and regression to the mean. It is important to note also that the placebo arm patients received conditioning with Methylprednisolone as part of the study protocol and it is not possible to exclude an effect from this, although the time period between the last dose of conditioning (2 weeks) and the fatigue assessment primary end point (12 weeks) would argue against this. Interestingly, the degree of response seen in the placebo group (36%) mirrors that seen in the placebo arm in recent trials of novel anti-pruritic therapy in PBC suggesting that this may represent a natural placebo response rate in this symptomatic condition (30). This observation will help with the design of future studies of symptomatic therapy in the condition. It is striking that any change in levels of patient reported outcomes was restricted to the primary outcome parameter, fatigue. It is also interesting to observe that the restriction of a placebo effect to the primary outcome symptom seen in this trial is mirrored in recent trials of anti-pruritus therapy, where a

placebo effect was seen for pruritus severity but not fatigue. It is also notable that a placebo effect for fatigue was absent in the POISE trial of second-line Obeticholic Acid therapy in PBC where symptom impact of treatment was not a principal focus of the trial pathology and fatigue severity was substantially lower in the study population than in the current fatigue-focused trial (median PBC-40 fatigue domain 24 compared with 42). One approach to mitigating the placebo effect in future trials in PBC might be to make the target more generic ("symptom burden" rather than a specific symptom). The very specific nature of the placebo effect also raises the question as to whether psychological interventions may have a value in at least mitigating the impact of symptoms in individual patients.

It is possible that our failure to recruit to the original planned group size may have introduced bias. Our experience was that exclusion criteria around possible chronic infection or malignancy resulted in a significant attrition rate in our cohort, reflecting largely age-related risk factors. In terms of study protocol it is possible that we missed an effect before the 3 month assessment, although the patient diary reporting in months 1 and 2 would argue against this

The variability in the apparent response to Rituximab was marked, raising the possibility that fatigue is heterogeneous in nature and that only a subgroup of patients, with a particular form of the disease, are responders to Rituximab. Since the design of the trial, it has become clear that a stratified approach to therapeutics in PBC may be highly effective; something which had previously been thought to be a major challenge using unselected patient approaches. The RITPBC trial used an unselected approach which may be a limitation. Again, subsequent to the design of the trial, it has become increasingly accepted

that central and peripheral fatigue have quite distinct characteristics in PBC. The underpinning data and study hypothesis in RITPBC were based on peripheral muscle abnormality (and peripheral fatigue). The study population showed, at baseline, appreciable levels of sleep disturbance and cognitive impairment which are features strongly associated with central rather than peripheral fatigue (31, 32). Given the proposed mechanism of action for Rituximab in the study it is possible that this is a disease group which may be unresponsive to the therapy. We do not believe that there is, at present, any rationale for further trials of Rituximab in PBC. It is worthy of note that as further data emerge regarding Rituximab and its use in other inflammatory conditions such as Primary Sjogrens Syndrome so confidence regarding any beneficial effect on fatigue reduces (33, 34) .

Bioenergetic abnormality was seen at baseline in the participants, mirroring the findings in the original studies. In terms of the bioenergetic model for fatigue in PBC, a number of conclusions can be drawn. The first is that the previously described bioenergetic abnormality is confirmed in this study in a cohort of highly fatigued patients. The second is that anaerobic threshold impairment is reduced in patients along with depletion of anti-PDC. Further analysis will be required to determine whether this link is causal, however the finding would be at least in keeping with the hypothesis that anti-PDC has a direct metabolic role increasing anaerobic metabolism. If this is indeed the case, it may be that the peak effect has been missed given that the peak in anti-PDC depletion was at 6 months, 3 months after the last anaerobic threshold assessment. The third conclusion is that the link between anti-PDC and anaerobic threshold, and muscle bioenergetic abnormality and perceived fatigue is limited. Change in post-exercise muscle pH with

Rituximab was limited, as was any change in fatigue. The apparent disconnect between anaerobic threshold on one hand and muscle bioenergetics abnormality on MR spectroscopy on the other would suggest that factors other than the balance between aerobic and anaerobic metabolism play predominant role in regulating muscle pH. The obvious candidate would be the capacity of muscle to handle protons and lactate; capacity which may be modified by addressing autonomic dysfunction (highly prevalent in PBC and postulated to impact on both transport of protons and lactate out of muscle cells and vascular outflow from muscle tissue) or through exercise therapy (which is known to increase proton/lactate transporter levels and to increase vascular outflow (35). Both approaches have evidence, albeit very limited, to suggest efficacy in PBC (36).

The trial was not designed or powered to explore the response of biochemical parameters to rituximab therapy, indeed a significant proportion of participants had normal serum biochemistry at baseline reflecting the lack of association between biochemical parameters of disease severity and fatigue severity. Interestingly, amongst the patients receiving rituximab therapy almost universal normalization of alkaline phosphatase was seen (with no comparable effect in the placebo group). Trials of immunotherapy as second-line treatment in Ursodeoxycholic acid (UDCA) under-responsive patients, have to date been unsuccessful (37). One potential explanation for this lack of efficacy could be that, unlike in our study, the biological agents may have been used “downstream” in the disease pathway, following proven failure of treatment with UDCA when cholestatic injury is established.

The conclusions of this study are firstly that trials of therapy for fatigue in PBC are highly acceptable, but the issue of a likely placebo effect will remain a real challenge that will

need to be addressed in future trial designs. The second is that the suppression in anaerobic threshold seen in PBC is apparently reversed by Rituximab therapy suggesting that there may be an antibody or other B-cell related process at play. The reduced post-exercise pH in muscle in PBC and impaired recovery which has been linked to fatigue was not, in contrast, improved by Rituximab suggesting that this is not linked to the reduced AT and suggesting that alternative approaches to improving muscle pH, including exercise therapy, should be explored.

References:

1. Lindor KD, Gershwin ME, Poupon R, Kaplan M, Bergasa NV, Heathcote EJ. Primary biliary cirrhosis. Hepatology 2009;50:291-308.
2. Goldblatt J, Taylor PJS, Lipman T, Prince MI, Baragiotta A, Bassendine MF, James OFW, et al. The true impact of fatigue in primary biliary cirrhosis: a population study. Gastroenterology 2002;122:1235-1241.
3. Jopson L, Jones DEJ. Fatigue in primary biliary cirrhosis: prevalence, pathogenesis and management. Digestive Diseases 2015;33:109-114.
4. Mells GF, Pells G, Newton JL, Bathgate AJ, Burroughs AK, Heneghan MA, Neuberger JM, et al. Impact of primary biliary cirrhosis on perceived quality of life: The UK-PBC national study. Hepatology 2013;58:273-283.
5. Dyson JK, Wilkinson N, Jopson L, Mells G, Bathgate A, Heneghan MA, Neuberger J, et al. The inter-relationship of symptom severity and quality of life in 2055 patients with primary biliary cholangitis. Alimentary pharmacology & therapeutics 2016;44:1039-1050.
6. Poupon RE, Chrétien Y, Chazouillères O, Poupon R, Chwalow J. Quality of life in patients with primary biliary cirrhosis. Hepatology 2004;40:489-494.
7. Nevens F, Andreone P, Mazzella G, Strasser SI, Bowlus C, Invernizzi P, Drenth JPH, et al. A placebo-controlled trial of obeticholic acid in primary biliary cholangitis. New England Journal of Medicine 2016;375:631-643.
8. Silveira MG, Gossard AA, Stahler AC, Jorgensen RA, Petz JL, Ali AH, Lindor KD. A randomized, placebo-controlled clinical trial of efficacy and safety: modafinil in the treatment of fatigue in patients with primary biliary cirrhosis. American journal of therapeutics 2017;24:e167-e176.
9. Talwalkar JA, Donlinger JJ, Gossard AA, Keach JC, Jorgensen RA, Petz JC, Lindor KD. Fluoxetine for the treatment of fatigue in primary biliary cirrhosis: a randomized, double-blind controlled trial. Digestive diseases and sciences 2006;51:1985-1991.
10. Meijer JM, Meiners PM, Vissink A, Spijkervet FKL, Abdulahad W, Kamminga N, Brouwer E, et al. Effectiveness of rituximab treatment in primary Sjögren's syndrome: a randomized, double-blind, placebo-controlled trial. Arthritis & Rheumatology 2010;62:960-968.

11. Rigby W, Ferraccioli G, Greenwald M, Zazueta-Montiel B, Fleischmann R, Wassenberg S, Ogale S, et al. Effect of rituximab on physical function and quality of life in patients with rheumatoid arthritis previously untreated with methotrexate. Arthritis care & research 2011;63:711-720.
12. Devauchelle-Pensec V, Morvan J, Rat A-C, Jousse-Joulin S, Pennec Y, Pers J-O, Jamin C, et al. Effects of rituximab therapy on quality of life in patients with primary Sjögren's syndrome. Clinical and Experimental Rheumatology-Incl Supplements 2011;29:6.
13. Fluge Ø, Bruland O, Risa K, Storstein A, Kristoffersen EK, Sapkota D, Næss H, et al. Benefit from B-lymphocyte depletion using the anti-CD20 antibody rituximab in chronic fatigue syndrome. A double-blind and placebo-controlled study. PloS one 2011;6:e26358.
14. Yeaman SJ, Kirby JA, Jones DEJ. Autoreactive responses to pyruvate dehydrogenase complex in the pathogenesis of primary biliary cirrhosis. Immunological reviews 2000;174:238-249.
15. Teoh KL, Rowley MJ, Zafirakis H, Dickson ER, Wiesner RH, Gershwin ME, Mackay IR. Enzyme inhibitory autoantibodies to pyruvate dehydrogenase complex in primary biliary cirrhosis: applications of a semiautomated assay. Hepatology 1994;20:1220-1224.
16. Hollingsworth KG, Newton JL, Robinson L, Taylor R, Blamire AM, Jones DEJ. Loss of capacity to recover from acidosis in repeat exercise is strongly associated with fatigue in primary biliary cirrhosis. Journal of hepatology 2010;53:155-161.
17. Hollingsworth KG, Newton JL, Taylor R, McDonald C, Palmer JM, Blamire AM, Jones DEJ. Pilot study of peripheral muscle function in primary biliary cirrhosis: potential implications for fatigue pathogenesis. Clinical Gastroenterology and Hepatology 2008;6:1041-1048.
18. Prentis J, Jones DE, Trenell MI, Snowden CP. P75 Impaired cardiorespiratory reserve in primary biliary cirrhosis patients undergoing liver transplant assessment. Gut 2011;60:A34-A35.
19. Myers RP, Swain MG, Lee SS, Shaheen AAM, Burak KW. B-cell depletion with rituximab in patients with primary biliary cirrhosis refractory to ursodeoxycholic acid. The American journal of gastroenterology 2013;108:933-941.
20. Jopson L, Newton JL, Palmer J, Floudas A, Isaacs J, Qian J, Wilkinson J, et al. RITPBC: B-cell depleting therapy (rituximab) as a treatment for fatigue in primary biliary cirrhosis: study protocol for a randomised controlled trial. BMJ open 2015;5:e007985.
21. Cohen SB, Emery P, Greenwald MW, Dougados M, Furie RA, Genovese MC, Keystone EC, et al. Rituximab for rheumatoid arthritis refractory to anti–tumor necrosis factor therapy: results of a multicenter, randomized, double-blind, placebo-controlled, phase III trial evaluating primary efficacy and safety at twenty-four weeks. Arthritis & Rheumatology 2006;54:2793-2806.
22. Jacoby A, Rannard A, Buck D, Bhala N, Newton JL, James OFW, Jones DEJ. Development, validation, and evaluation of the PBC-40, a disease specific health related quality of life measure for primary biliary cirrhosis. Gut 2005;54:1622-1629.
23. Zigmond AS, Snaith RP. The hospital anxiety and depression scale. Acta psychiatrica scandinavica 1983;67:361-370.
24. Johns MW. Sleepiness in different situations measured by the Epworth Sleepiness Scale. Sleep 1994;17:703-710.
25. Schrezenmaier C, Gehrking JA, Hines SM, Low PA, Benrud-Larson LM, Sandroni P. Evaluation of orthostatic hypotension: relationship of a new self-report instrument to laboratory-based measures. In: Mayo Clinic Proceedings; 2005: Elsevier; 2005. p. 330-334.
26. Bruce B, Fries JF. The health assessment questionnaire (HAQ). Clinical and experimental rheumatology 2005;23:S14.
27. Broadbent DE, Cooper PF, FitzGerald P, Parkes KR. The cognitive failures questionnaire (CFQ) and its correlates. British journal of clinical psychology 1982;21:1-16.
28. Tsuda M, Moritoki Y, Lian ZX, Zhang W, Yoshida K, Wakabayashi K, Yang GX, et al. Biochemical and immunologic effects of rituximab in patients with primary biliary cirrhosis and an incomplete response to ursodeoxycholic acid. Hepatology 2012;55:512-521.
29. Dhirapong A, Lleo A, Yang GX, Tsuneyama K, Dunn R, Kehry M, Packard TA, et al. B cell depletion therapy exacerbates murine primary biliary cirrhosis. Hepatology 2011;53:527-535.

30. Hegade VS, Kendrick SFW, Dobbins RL, Miller SR, Thompson D, Richards D, Storey J, et al. Effect of ileal bile acid transporter inhibitor GSK2330672 on pruritus in primary biliary cholangitis: a double-blind, randomised, placebo-controlled, crossover, phase 2a study. The Lancet 2017;389:1114-1123.
31. Newton JL, Gibson GJ, Tomlinson M, Wilton K, Jones D. Fatigue in primary biliary cirrhosis is associated with excessive daytime somnolence. Hepatology 2006;44:91-98.
32. Newton JL, Hollingsworth KG, Taylor R, El-Sharkawy AM, Khan ZU, Pearce R, Sutcliffe K, et al. Cognitive impairment in primary biliary cirrhosis: symptom impact and potential etiology. Hepatology 2008;48:541-549.
33. Bowman SJ, Everett CC, O'Dwyer JL, Emery P, Pitzalis C, Ng WF, Pease CT, et al. Randomized Controlled Trial of Rituximab and Cost-Effectiveness Analysis in Treating Fatigue and Oral Dryness in Primary Sjögren's Syndrome. Arthritis & Rheumatology 2017.
34. Letaief H, Lukas C, Barnetche T, Gaujoux-Viala C, Combe B, Morel J. Efficacy and safety of biological DMARDs modulating B cells in primary Sjögren's syndrome: Systematic review and meta-analysis. Joint Bone Spine 2017.
35. Pilegaard H, Domino K, Noland T, Juel C, Hellsten Y, Halestrap AP, Bangsbo J. Effect of high-intensity exercise training on lactate/H+ transport capacity in human skeletal muscle. American Journal of Physiology-Endocrinology And Metabolism 1999;276:E255-E261.
36. Jones DEJ, Sutcliffe K, Pairman J, Wilton K, Newton JL. An integrated care pathway improves quality of life in primary biliary cirrhosis. QJM: An International Journal of Medicine 2008;101:535-543.
37. Hirschfield GM, Gershwin ME, Strauss R, Mayo MJ, Levy C, Zou B, Johanns J, et al. Ustekinumab for patients with primary biliary cholangitis who have an inadequate response to ursodeoxycholic acid: A proof-of-concept study. Hepatology 2016;64:189-199.

Author names in bold designate shared co-first authorship.

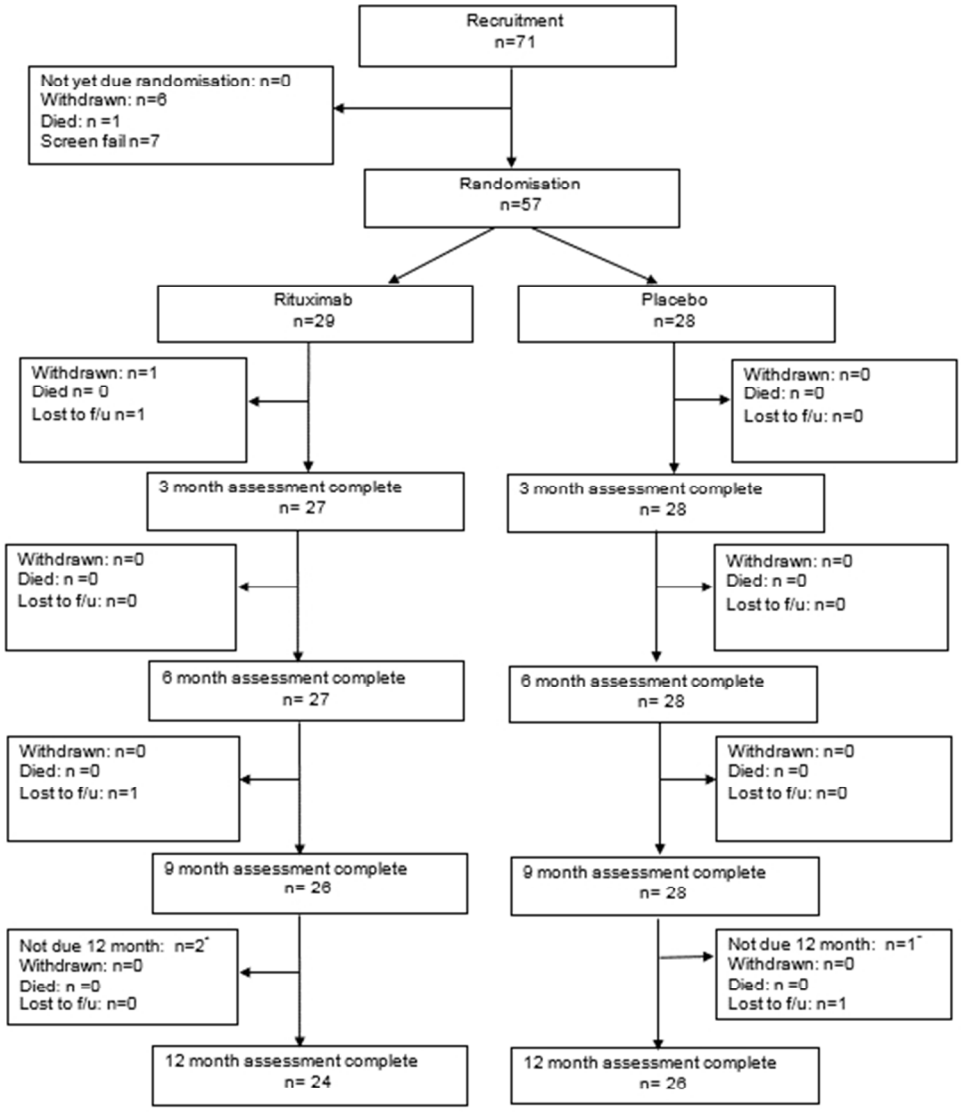
Figure Legends:

Figure 1: Trial CONSORT diagram

Figure 2: PBC-40 fatigue scores at baseline, 3 months (primary end-point) and 6, 9 and 12 months. In the Rituximab and Placebo groups (primary endpoint)

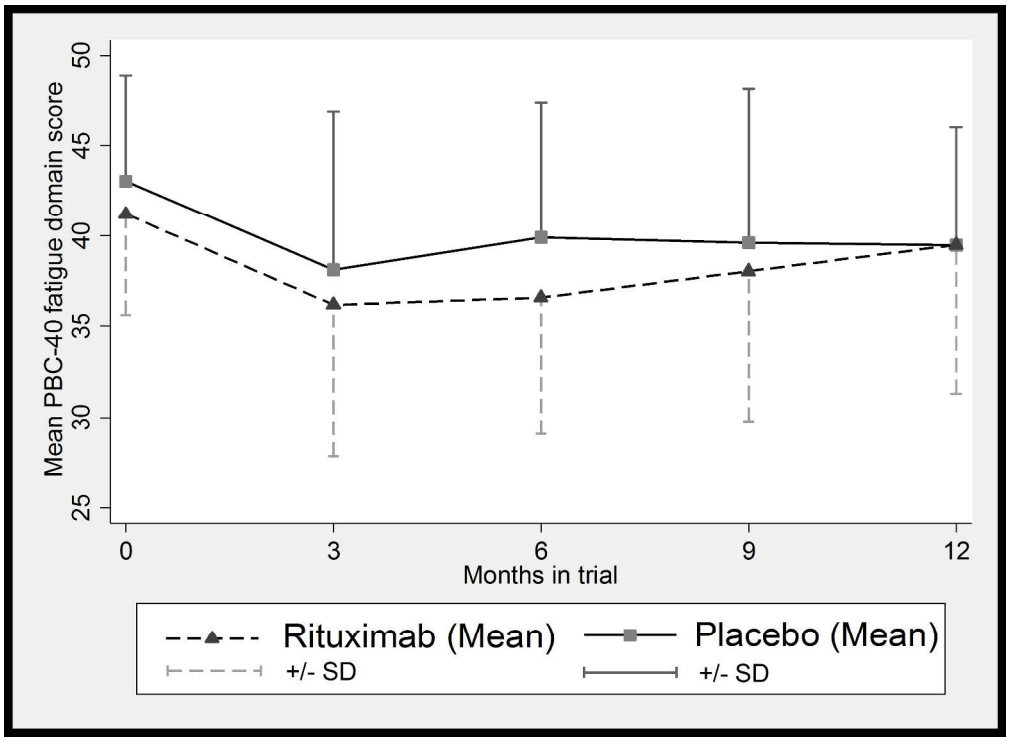
Figure 3: Immunological changes seen in the Rituximab and Placebo-treated groups. **a)** B-cells as a proportion of the total CD45+ population. **b)** Serum anti-PDC level as a proportion of the baseline, pre-treatment value (secondary end-points).

Figure 4: Impact of Rituximab and Placebo on bioenergetics function in terms of **a)** Anaerobic Threshold and **b)** Minimum muscle pH following controlled exercise (secondary end-points).

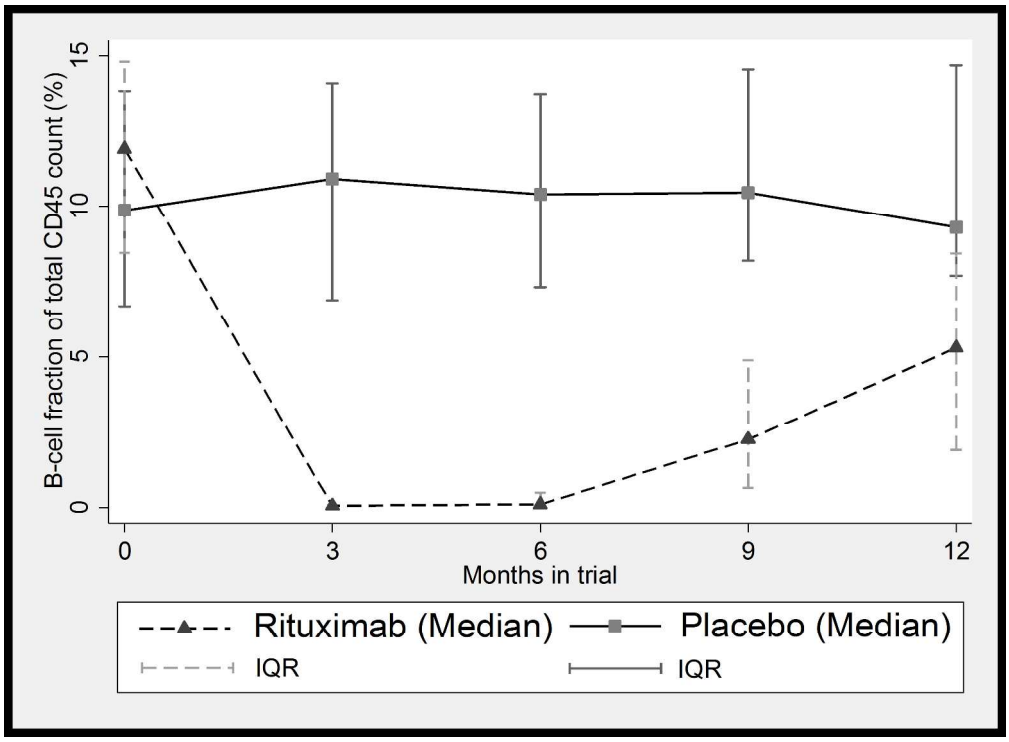


* Trial was terminated early on 12th Sept 2016 so these patients forewent their 12 month follow-up visits which were due after this date

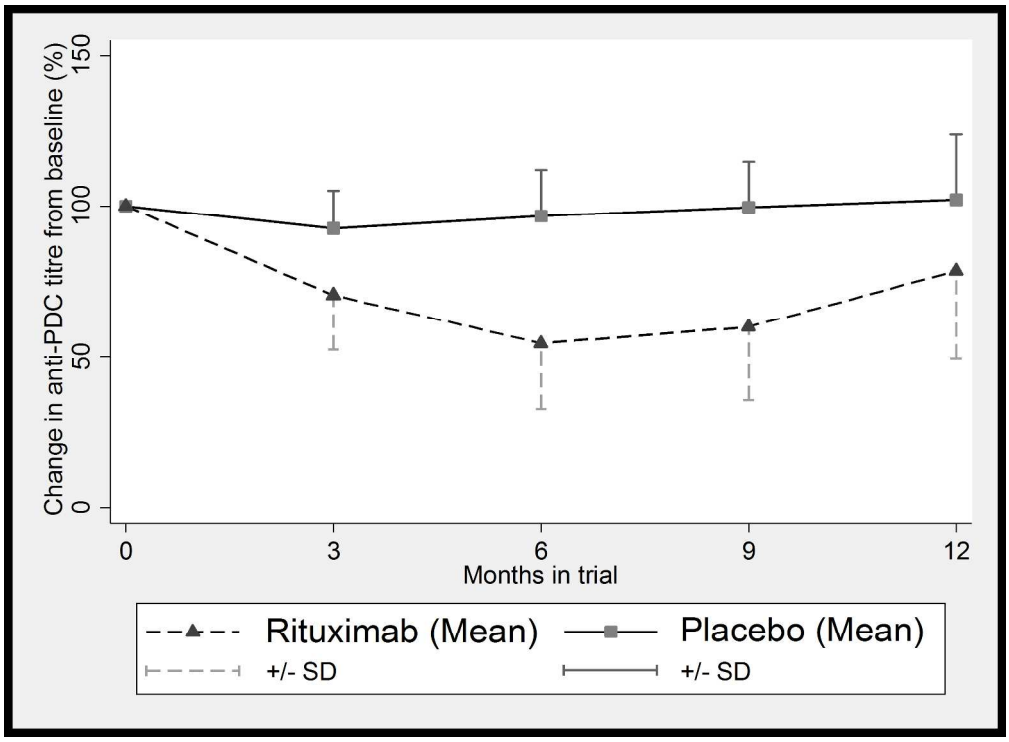
47x57mm (300 x 300 DPI)



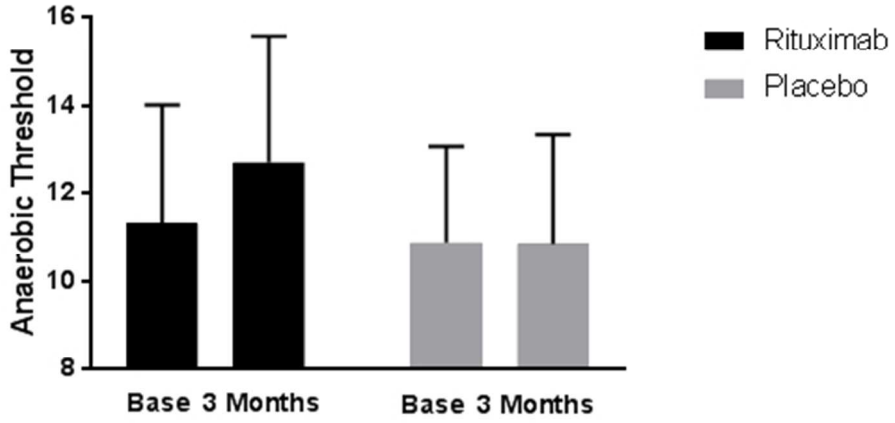
284x208mm (300 x 300 DPI)



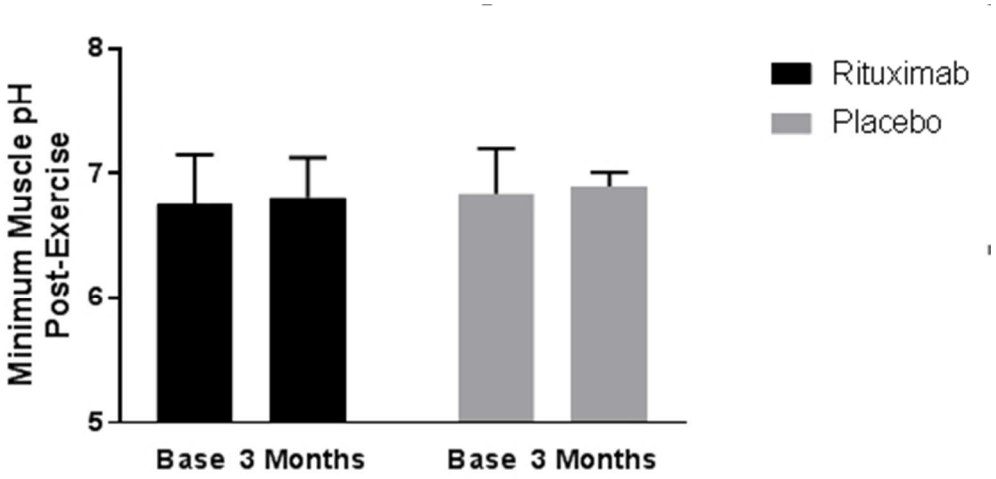
284x208mm (300 x 300 DPI)



284x208mm (300 x 300 DPI)



45x23mm (300 x 300 DPI)



43x21mm (300 x 300 DPI)

Table 1:

	Rituximab (n=29)		Placebo (n=28)	
	Median (IQR)	**Range**	**Median (IQR)**	**Range**
Age in years	55.9 (48.8-60.0)	34.0-66.7	53.3 (49.9-58.8)	39.2-72.1
Alcohol consumption (units per week-drinkers)	4 (2-8)	1-12	4 (2-12)	1-14
Alcohol consumption (units per week -ALL)	1 (0-4)	0-12	0 (0-1.5)	0-14
BMI	28.7 (24.5-30.5)	19.8-40.5	26.7 (22.9-30.7)	18.7-43.5
	n	**(%)**	**n**	**(%)**
Sex: Female	28	96.5	27	96.5
Ethnicity:				
White	27	96.5	28	100
Non-white	1	3.5	0	0
Smoking status:				
Never	16	55	12	43
Past	7	24	8	28.5
Current	6	21	8	28.5
Managed by Newcastle	20	74	19	68
UDCA use: Yes	24	89	27	96.5
If yes: Responder	19	79	16	59
Baseline Lab Parameters:				
	Mean (SD)	**Range**	**Mean(SD)**	**Range**
Hemoglobin (g/L)	127.8 (10.9)	104-152	131.0 (8.2)	110-151
WBC (10^9/L)	5.8 (1.5)	3.7-8.6	6.3 (1.4)	3.6-8.8
Platelet count (10^9/L)	274.6 (59.1)	132-397	276.9 (91.8)	105-444
PT (secs)	10.9 (0.6)	10-12	11.0 (0.7)	10-13
Bilirubin (mmol/L)	7.3 (3.9)	3-22	7.4 (3.7)	3-21
Alkaline phosphatase (U/L)	156.6 (72.4)	52-339	217.1 (166.8)	45-642
ALT (U/L)	48.4 (33.0)	10-150	50.2 (29.2)	11-135
AST (U/L)	45.0 (25.8)	19-128	46.6 (24.9)	14-105
Albumin (G/L)	44.7 (2.1)	40-49	43.1 (2.2)	37-47
GGT (U/L)	140.8 (157.7)	22-559	197.4 (224.5)	12-853
APTT (secs)	34.0 (5.4)	26-52	32.8 (3.2)	28-43
CRP	6.9 (5.5)	5-33	7.2 (4.8)	5-21
Baseline Immunoglobulin levels:				
	Mean (SD)	**Range**	**Mean(SD)**	**Range**

Total IgG:	12.5 (3.4)	7.5-22.2	11.8 (2.55)	7.6-17.9
Total IgM:	3.7 (2.1)	0.9-8.7	3.2 (2.1)	0.5-10.3
Total Ig level:	18.6 (5.0)	11.4-32	17.1 (4.4)	9.8-28.3
Baseline UKPBC 40 Fatigue and 10 year UK PBC risk score:				
	Mean (SD)	**Range**	**Mean(SD)**	**Range**
PBC-40 Fatigue (*11-55*):	41.2 (5.5)	32-54	43.0 (5.9)	31-54
	Median (IQR)	**Range**	**Median (IQR)**	**Range**
UK PBC risk score at 10 years	1.26 (0.94-1.74)	0.21-3.55	1.75 (1.12-3.04)	0.20-12.90